Contextual fear memory generalization

To address the question of how contextual fear contributed to cued fear generalization, pre-CS freezing (180 s) in the training and testing environments across testing phases (Time: pre-training, pre-Recent CS, pre-Remote CS) was analyzed first. In the within-subjects design, repeated measures ANOVA revealed a significant main effect of Time ($F[2, 108]=40.0$; $p<.001$), but no Time X kHz interaction. There was greater overall freezing from Pre-CS/US (training) (11.4 ± 1.7) to Pre-CS (testing) (33.1 ± 2.5) at the recent time frame ($p<.001$; 190% increase), indicating generalized fear of the new context (Figure S2). There was also greater freezing at the Pre-CS remote time frame (50.2 ± 3.6) compared with the Pre-CS recent time frame ($p<.001$; 51.7% increase) (Figure S2). These data indicate that contextual freezing increased over time. In the between-subject design, repeated measures revealed a significant main effect of Time ($F[1, 155]=33.9$; $p<.001$), but not a Time x kHz interaction. There was greater freezing from Pre-CS/US (12.1 ± 1.8) to Pre-CS (30.3 ± 2.4) at the recent time frame ($p<.001$; 147% increase) and greater freezing at the Pre-CS remote time frame (51.5 ± 2.7) compared with the Pre-CS recent time frame ($p<.001$; 64.7% increase) (Figure S2). Overall, these results show robust fear memory generalization to the context 1 d following learning and increased generalization over 30 d.

Experiment 3: No context pre-exposure

To test the influence of a change in context (Testing chamber) at the recent time point (i.e., the between-subjects design) on cued and context generalization at a remote time point, we added another group that was not exposed to the novel context at a recent time point. Results revealed a significant degree of generalization of both the 2-kHz ($p<.05$) and 3-kHz ($p<.05$) stimuli. However, analysis of contextual Pre-CS to CS freezing revealed no statistical differences. A comparison of pre-CS freezing levels in the BS remote group and the No context group revealed significantly more freezing in the No context group ($F[1,91]=19.5$; $p<.001$).

Together, these data suggest that exposing mice to a novel context at a recent time point following learning reduces background context generalization over time (Figure S3).

Arc expression: MANOVA (main effect of Time)

MANOVA also revealed main effects of Time (V=.68, F[10, 106] = 5.5; p<.001). Follow-up ANOVAs revealed effects of Time across the PL (F[2,56] = 6.9; p=.002), TeA ([2,56] = 5.8; p=.005), and IL ([2,56]= 8.2; p=.001). When comparing the recent to BS remote time points, there was a significant decrease in *Arc* expression in the IL only (p=.049) (Figure 2). These data suggest that cued fear memory storage over time is associated with relatively stable *Arc* expression, except for a reduction in *Arc* expression in the IL. In contrast to the recent-remote BS comparison, a recent-WS remote comparison revealed a significant increase in *Arc* expression in the PL (p=.03), TeA (p=.01) (Figure 3), and significant decrease in *Arc* expression in the IL (p=.003). Finally, comparisons of the Remote groups revealed significantly greater *Arc* expression in the WS remote group in the PL (p=003), TeA (p=.035), and AIP (p=.03) (Figure 4). Notably, there was no effect of Time in the LAd, indicating a high degree of stability for fear memory retrieval-induced *Arc* expression in the LAd over time (Figure 5).

Discriminant Function Analysis (DA)

Next, we applied discriminant function analysis (DA) to understand the nature of the relationship among cortico-amygdala regions expressing *Arc* and how the relationship predicts memory performance (discrimination or generalization) at different time points following learning. At the recent time point, results of DA showed the first discriminant function (DF1) segregated kHz (Λ=.2, X^2[12]= 26.2; p=.01) and accounted for 70.2% of the variance in the data set (Figure S5). No other functions discriminated the groups, indicating a single underlying dimension of the data set discriminated kHz frequency at the recent time point. Examination of the structure matrix for DF1 revealed the relative contribution of the LAd (.59), followed by the PL (.48) discriminated *Arc* expression in the 5-kHz group from 3-kHz and Control conditions.

This result confirms a leading role for LAd and PL in fear memory expression. The relationship between the variate scores and the groups was plotted in a scatter plot (Figure S5) and variate scores statistically compared between groups (kHz frequency) using ANOVA. ANOVA on the variate scores ($F[2,19]=16.9$; $p<.001$) confirmed greater scores for DF1 in the 5-kHz relative to 3-kHz ($p=.005$) and control ($p<.001$) conditions. This result suggests a pattern of synaptic plasticity in the LAd/PL that is engaged early in the discrimination of conditioned fear stimuli.

In the remote between-subject design, results of DA reveal the first discriminant function (DF1) segregated kHz ($\Lambda=.11$, $X^2[12]= 36.6$; $p<.001$) and accounted for 95.4% of the variance in the data set. No other discriminant functions predicted group membership, indicating a single underlying dimension discriminated kHz frequency at the remote time point in the between-subjects design. The structure matrix for DF1 revealed contributions of the AIP (.44), followed by the IL (.29) discriminated the groups (Figure S5). ANOVA on the variate scores for DF1 ($F[2,19]=57.4$; $p<.001$) revealed greater scores in the 5-kHz ($p<.001$) and 3-kHz ($p<.001$) groups compared to the control condition. This result indicates that a pattern of plasticity in AIP and IL cortical regions underlies fear memory generalization at remote retention intervals following learning.

In the remote within-subject design, results of DA showed only the first discriminant function (DF1) segregated kHz frequency ($\Lambda=.19$, $X^2[12]= 25.1$; $p=.015$) and accounted for 91.1% of the variance in the data set. Examination of the structure matrix for DF1 revealed a nearly identical contribution of *Arc* expression in the PL (.46) and LAd (.458) discriminated the groups. ANOVA on the variate scores for DF1 ($F[2,18]=26.3$; $p<.001$) showed greater scores for in the 5-kHz ($p<.001$) and 3-kHz ($p<.001$) groups compared to the control condition. These results suggest that at a remote time point following learning and cued fear memory reactivation, the PL and LAd exhibit plasticity related to cued fear memory discrimination.

Arc-positive cell density analysis

MANOVA revealed a significant interaction of Time X kHz on the density of Arc+ cells in the LA, PL and TeA *(V*=.47, *F*[12, 162]=.2.5; *p*=.005). Follow-up univariate ANOVAs revealed significant interactions of Time x kHz on the PL (*F*[4,54] = 4.33; *p*=.004) and TeA (*F*[4,54] = 3.6; *p*=.01). In the PL, post hoc analysis at the recent time point revealed greater Arc expression in the 5-kHz relative to both 3-kHz (p=.006) and no tone control groups (p = .007). There were no differences between the 3-kHz and 5-kHz conditions. At the remote time point in the between-subjects design (remote-BS), post hoc analysis revealed no significant differences between groups. At the remote time point in the within-subjects design (remote-WS), post hoc analysis indicated greater Arc expression at the 3-kHz frequency relative to the no tone control (*p*=.01) and 5-kHz (*p*=.03) groups. There was no difference in Arc expression between the 5-kHz and no tone control group. In the TeA, post hoc analysis at the recent time point indicated a reduction in Arc expression at the 3-kHz relative to the 5-kHz (*p*=.03) frequency and no tone control groups (*p*=.002). At the remote-BS time point, post hoc analysis revealed no significant differences between groups. At the remote WS time point, post hoc analysis revealed no significant differences between groups.

MANOVA also revealed main effects of Time across the LA (p=.01), PL (p<.001) and TeA (p<.001), with a significant decrease in the density of Arc expression following retrieval from recent to remote time points. There was also a main effect of kHz for the LA only (F[2, 17] = 5.2; p=.016). In the LA, there was increase Arc expression in the 5-kHz group compared with the no tone control (p=.006). There were a nearly significant increase in Arc expression in the 5-kHz group compared with the 3-kHz group (p=.06). There was no differences in Arc expression between the 3-kHz and no tone control group.